Amendments to the Specification:

Pursuant to 37 C.F.R. § 1.57 please replace the paragraph on page 1 that begins with line 4 and runs through line 5, with the following paragraph:

This application claims priority from U.S. Provisional Application Serial No. 60/171,459 filed December 22, 1999 the disclosure of which is hereby incorporated by reference in its entirety.

Pursuant to 37 C.F.R. § 1.57 please insert the following paragraph on page 5 after the paragraph ending with line 4 and before the paragraph beginning with line 5:

Figure 7 is a comparative bar graph depicting the antibody titers obtained from mice immunized with vaccines comprising: 1.) plasmid containing protective antigen (pCPA); 2.) plasmid containing a mutant lethal factor (pCLF4); and 3.) a combination of pCPA and pCLF4.

Pursuant to 37 C.F.R. § 1.57 please insert the following paragraphs on page 19 after the paragraph ending with line 30 and before the paragraph beginning with line 31:

Example 4

Plasmid Constructs

The eukaryotic expression plasmid, pCI (Promega Inc.), was used to evaluate whether the genes encoding the lethal factor (LF) and protective antigens (PA) could be used in formulations for construction of a DNA-based vaccine against the lethal effects of anthrax toxin. The vector, pCI (Promega Inc.), utilizes the cytomegalovirus intermediate-early promoter/enhancer region and the SV40 late polyadenylation signal for efficient expression of encoded genes. The PA gene, nucleotides encoding amino acids 204 to 764 of SEQ ID NO:4, that was cloned into the vector includes the wild-type PA gene sequence from a furin cleavage site to the end of the gene. This PA sequence starts at amino acid 204, valine, after the ATG start codon and ends at the end of PA. The LF gene, nucleotides encoding amino acids 83 to 283 of SEQ ID NO:2, that was cloned into the vector is a mutant form of LF and the clone contains the portion of the LF gene which encodes amino acids 83 to 283. In addition, there is a mutation at amino acid 170 of SEQ ID NO:2, which changes TAT (Tyr) to TGT (Cys).

Immunization

Female BALB/c ByJ mice, 4-5 weeks old (Jackson Laboratories, Bar Harbour, ME) were

immunized via gene gun inoculation on days 0, 14, and 28 with 0.5 ug of either pVR1020 (control), pCLF4, pCPA, or pCLF4 and pCPA, coated onto 1 micron gold beads via a previously described protocol (Bio-Rad Helios Gene Gun System Instruction Manual, p. 19-23). Sera samples were taken from three mice from each group on day 42. Mice were challenged on day 57 with 5 X LD50 of wild-type PA plus LF injected i.v. into the tail veins of immunized or control mice.

ELISA assay for anti-PA and anti LF antibodies

Antibodies raised against PA and LF were analyzed by ELISA as previously described. Briefly, Immulon 2 plates (Dynatech Laboratories, Inc., Chantilly, VA) were coated with 100 ng native PA or LF in O.1M carbonate buffer, pH 9.6. Plates were washed and blocked with 1% BSA in TBS. Serum samples were serially diluted in TBS, 0.05% Tween-20 and added to the plate; anti-mouse Ig conjugated to horse radish peroxidase (Sigma Chemical Co., St. Louis, MO) was used as the secondary antibody. The presence of bound antibody was detected using ABTS substrate (Zymed, S. San Francisco, CA) and absorbance read at 405 nm on a Bio-Rad Model 550 plate reader. Antibody titers were defined as the highest serum dilution that resulted in an absorbance value two times greater than a non-immune serum control with a minimum value of 0.05.

Results

Immunization with PA and/or mutant LF results in neutralizing antibodies. It was important to determine whether DNA-based immunization with pCPA and/or pCLF4 would result in the production of a protective immune response *in vivo*. High titered (Figure 7), specific antisera was obtained from the immunizations. Significantly the titers of the serum from the mice immunized with both pCPA and pCLF4 show a titer that is at least 2 - 4 times higher than serum titers from mice immunized separately with either of the genes alone. The results of the toxin challenge experiment (Table 4) illustrate that DNA-based immunization with the plasmids containing the genes that encode PA (pCPA), mutant LF (pCLF4), or a combination of the two plasmids (pCPA and pCLF4) results in a protective immune response when immunized animals are challenged with wildtype PA plus LF (which constitutes the lethal toxin). Significantly, animals immunized with pCPA and/or pCLF4 were completely protected against challenge with a significant amount of PA plus LF (5 x LD50). These results demonstrate the efficacy of DNA-

based immunization and serve to illustrate that protection against the effects of *B. anthracis* infection can be achieved by immunizing with the PA and/or LF genes or derivatives thereof.

Table 4. Vaccination with pCPA, pCLF4, or a combination of them confers protection against lethal anthrax challenge.

		Immunized Mice			
Challenge Dose	LD_{50}	Vector	pPA	pLF4	pLF4 + pPA
60 ug PA, 25 ug LF4	5	0/3	3/3	3/3	4/4

A mixture of PA (60 ug/mouse) and LF (25 ug/mouse) was injected i.v. into immunized or vector treated BALB/c mice. Values shown are number of survivors/number challenged.